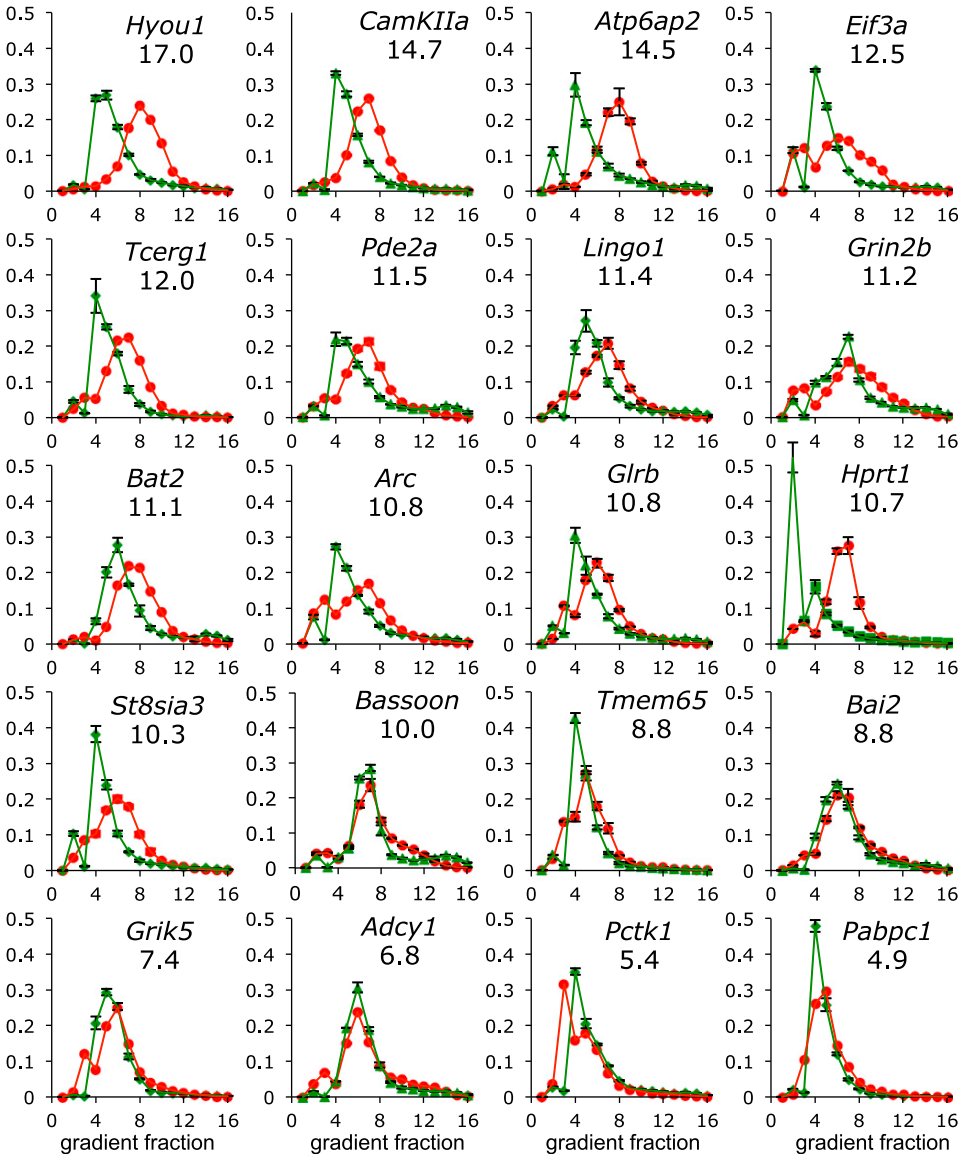

Figure S5. EDTA Profiles of FMRP Target and Nontarget Transcripts after Run-Off, Related to Figure 5

EDTA treatment of polyribosomes in the IVT_{EBP} assay after puromycin run-off and kcRNA treatment. Following puromycin run-off in the presence of kcRNA (red) or kcRNA plus 30 mM EDTA (green) samples were run on 20%–50% sucrose gradients, and qRT-PCR was used to measure the levels of the indicated mRNAs in each fraction. Individual mRNAs and their "ribosome loading" score (Table S6) are indicated on each graph. Ribosome loading is a measure of the total number of ribosomes associated with transcripts on polyribosome gradients before run-off. Graphs are arranged in order of descending "ribosome loading," and illustrate the dependence of the number of retained ribosomes (the difference between the red and green graphs) on the number of ribosomes initially associated with each transcript.

Additional comments: Examination of the mRNA distribution for most mRNAs after puromycin run-off, even in the presence of kcRNA decoy, suggested that most transcripts still harbor retained ribosomes. A priori, dogma predicts that free mRNA without retained ribosomes should be in the lightest fractions of the sucrose gradient (Mathews et al., 2007). To address this issue, we examined the distribution of 10 target and 10 non-target mRNAs, comparing the distribution of ribosome-depleted mRNAs (EDTA-treated, green) with their distribution after full run-off (red) in the IVT_{EBP} assay as described above. Surprisingly, in most cases mRNA stripped of ribosomes peaked in fractions 4-5, contrasting with prior analysis suggesting that mRNPs should be in fractions 1-2 of similar gradients. While we do not fully understand this discrepancy, we note that these prior studies were focused on analysis of relatively short housekeeping genes that are likely under little regulatory control and may therefore be associated with fewer regulatory mRNA binding proteins (e.g., see (Fagan et al., 1991)). Consistent with this observation, we found that *Hprt1*, a short housekeeping transcript, peaks after EDTA ribosomal stripping in fraction 2.

More generally, most transcripts show a profile with peaks varying between fractions 5 and 8 after puromycin run-off, and show additional shifts toward fraction 4 after EDTA stripping. A few transcripts (*Bsn, Tmem65, Bai2, Grik5, Adcy1, Pctk1, and Pabpc1*) are largely fully run-off (show little/no difference in profiles after EDTA treatment) in the IVT_{EBP} assay. For the remaining transcripts, the number of puromycin insensitive ribosomes remaining after run-off is not related to CDS length, transcript abundance, to the final location of ribosome-depleted mRNA migration, nor, importantly, to whether the transcript is an FMRP target (see Table

S6). In contrast, the migration is very well-correlated with the number of ribosomes associated with the transcript in the steady state ("ribosome-loading"; a score derived from CHX-treated gradients; e.g., Figure 4, yellow lines). We conclude that the more ribosomes a transcript is loaded with in the steady-state, the more ribosomes will remain after puromycin run-off in the IVT_{EBP} system. While the mechanism underlying this phenomenon is unclear, it *is* clear that it is independent of whether the transcript is an FMRP target.